

1 **Original article**

2 **SARS-CoV-2 Spike protein promotes hyper-inflammatory response that can be**
3 **ameliorated by Spike-antagonistic peptide and FDA-approved ER stress and MAP**
4 **kinase inhibitors *in vitro***

5
6 Alan C-Y. Hsu^{1,2} †, Guoqiang Wang³, Andrew T. Reid^{1,2}, Punnam Chander. Veerati^{1,2},
7 Prabuddha S. Pathinayake^{1,2}, Katie Daly^{1,2}, Jemma R. Mayall^{1,2}, Philip M. Hansbro^{4,5}, Jay C.
8 Horvat^{1,2}, Fang Wang³, and Peter A. Wark^{1,2,6}.

9
10 ¹ Priority Research Centre for Healthy Lungs, The University of Newcastle, Newcastle, New
11 South Wales 2305, Australia

12 ² Viruses, Infection / Immunity, Vaccines and Asthma, Hunter Medical Research Institute,
13 Newcastle, New South Wales 2305, Australia

14 ³ Department of Pathogen Biology, College of Basic Medical Science, Jilin University,
15 Changchun 130021, China.

16 ⁴ School of Life Sciences, University of Technology Sydney, New South Wales 2007,
17 Australia

18 ⁵ Centenary UTS Centre for Inflammation, Centenary Institute, New South Wales 2050,
19 Australia

20 ⁶ Department of Respiratory and Sleep Medicine, John Hunter Hospital, Newcastle, New
21 South Wales 2305, Australia

22
23 † Correspondence: Dr Alan Hsu, Ph.D.

24 Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute, Lot 1
25 Kookaburra Circuit, New Lambton Heights, Newcastle, NSW 2305, Australia.

26 Email: Alan.Hsu@newcastle.edu.au.

27

28 The authors have declared that no conflict of interest exists.

29

30

31 **Summary**

32 SARS-CoV-2 infection causes an inflammatory cytokine storm and acute lung injury.
33 Currently there are no effective antiviral and/or anti-inflammatory therapies. Here we
34 demonstrate that 2019 SARS-CoV-2 spike protein subunit 1 (CoV2-S1) induces high levels
35 of NF- κ B activations, production of pro-inflammatory cytokines and mild epithelial damage,
36 in human bronchial epithelial cells. CoV2-S1-induced NF- κ B activation requires S1
37 interaction with human ACE2 receptor and early activation of endoplasmic reticulum (ER)
38 stress, and associated unfolded protein response (UPR), and MAP kinase signalling
39 pathways. We developed an antagonistic peptide that inhibits S1-ACE2 interaction and
40 CoV2-S1-induced productions of pro-inflammatory cytokines. The existing FDA-approved
41 ER stress inhibitor, 4-phenylbutyric acid (4-PBA), and MAP kinase inhibitors, trametinib and
42 ulixertinib, ameliorated CoV2-S1-induced inflammation and epithelial damage. These novel
43 data highlight the potentials of peptide-based antivirals for novel ACE2-utilising CoVs, while
44 repurposing existing drugs may be used as treatments to dampen elevated inflammation and
45 lung injury mediated by SARS-CoV-2.

46

47

48 **Keywords:**

49 SARS-CoV-2, COVID-19, Coronavirus, Inflammation, Endoplasmic Reticulum stress

50

51

52 **Introduction**

53 The emergence of a novel SARS-coronavirus in late 2019 (SARS-CoV-2; previously known
54 as 2019-nCoV), and the CoV disease (COVID)-19 it causes, has led to a devastating
55 pandemic of the 21st century. SARS-CoV-2 belongs to the beta-coronavirus genus with
56 approximately 79.5% sequence homology to the SARS-CoV that emerged in 2002 (Wang et
57 al., 2020b). Similar to SARS-CoV (2002), this novel CoV also utilises angiotensin converting
58 enzyme (ACE)2 as its host receptor to mediate membrane fusion and virus entry and viral
59 replication (Zhou et al., 2020). SARS-CoV-2 spike protein contains two subunits, subunit 1
60 (S1) and S2, that mediates viral attachment to ACE2 and membrane fusion, respectively. The
61 receptor-binding domain (RBD) of S1 is the critical region of the spike protein for ACE2
62 binding.

63

64 Human bronchial epithelial cells are both susceptible and permissive to CoV infection and
65 replication, and the innate immune responses produced by which are critical in the early
66 containment of infection and spread. Viral infection results in the activations of several
67 pattern recognition receptors (PRRs) including retinoic acid-inducible gene I like receptors
68 (RLRs; RIG-I and melanoma differentiation associated protein 5 (MDA-5))(Hayman et al.,
69 2019; Saito and Gale, 2008), and toll-like receptors (TLRs; TLR3 and TLR7)(Alexopoulou et
70 al., 2001; Diebold et al., 2004; Le Goffic et al., 2007). Recognition of viral RNAs by these
71 PRRs leads to activation of transcription factor nuclear factor kappa-light-chain-enhancer of
72 activated B cells (NF- κ B), which facilitates the expression of pro-inflammatory cytokines
73 such as interleukin (IL)-6 and IL-1 β . These cytokines recruit and activate important immune
74 cells including macrophages and neutrophils, which further promote inflammation and
75 contain viral spread (Guan et al., 2020) (Wang et al., 2008). Patients with severe COVID-19
76 have been shown to have develop enhanced systemic inflammatory responses (aka. cytokine
77 storm), and acute lung injury and acute respiratory distress syndrome (ARDS) (Huang et al.,
78 2020; McGonagle et al., 2020; Mehta et al., 2020). This storm is characterised by heightened
79 levels of IL-6, tumor necrosis factor- α (TNF- α), and C-C motif chemokine ligand (CCL)2.
80 Currently there are no specific antiviral drugs available or anti-inflammatory drugs that have
81 been shown to influence clinical outcomes for people with COVID-19. A broad-spectrum
82 antiviral drug remdesivir is currently being used for COVID-19 through compassionate use
83 requests as well as in clinical trials in the US and China. The anti-malarial drug
84 hydroxychloroquine is also under several clinical trials, although preliminary reports have not
85 shown beneficial effects (Magagnoli et al., 2020; Mahevas et al., 2020).

86

87 Here we demonstrate that the SARS-CoV-2 spike protein S1 (CoV2-S1) or RBD (CoV2-
88 RBD) alone stimulates more pronounced production of pro-inflammatory cytokines as well
89 as factors associated with epithelial damage, compared with the S1 and RBD of SARS-CoV
90 (CoV-S1 and -RBD, respectively). This heightened inflammatory response requires S1 and
91 ACE2 interaction and is primarily driven by early endoplasmic reticulum (ER) stress and its
92 adaptive unfolded protein response (UPR), as well as activation of mitogen-activated protein
93 (MAP) kinase signalling pathways. The early induction of ER-UPR results in activation of
94 MAP kinase, and both pathways synergistically leads to NF- κ B activation and production of
95 the pro-inflammatory cytokines IL-6, IL-1 β , TNF α , and CCL2, the latter three of which have
96 all been shown to contribute to acute lung injury(Kolb et al., 2001) (Sheridan et al., 1997).

97

98 Since CoV2-S1 induces NF- κ B activation *via* its interaction with ACE2 and early activations
99 of ER-UPR and MAP kinase signalling, we designed a series of CoV2-S1-antagonistic
100 peptides and identified a peptide, designated AP-6, that inhibits S1-ACE2 interaction. We
101 show that CoV2-S1-mediated inflammatory response are not only ameliorated by AP-6, but
102 the heightened inflammatory responses are also inhibited by an FDA-approved ER stress
103 inhibitor 4-phenylbutic acid (4-PBA), and MAP kinase inhibitors trametinib (GSK1120212)
104 and ulixertinib (BVD-523).

105

106 Taken together, these data shed light on how the spike protein of SARS-CoV-2 may
107 contribute to the exaggerated inflammatory responses and pathology observed in those with
108 severe COVID-19. The specific antagonistic peptide that specifically target S1 may serve as
109 an antiviral against SARS-CoV-2. We also showed how existing, pathway specific, FDA-
110 approved drugs could be re-purposed and immediately deployed to reduce infection-induced
111 symptoms and pathologies that are primarily driven by exaggerated inflammatory responses.

112

113 **Results**

114 **SARS-CoV-2 spike protein S1 and RBD induces elevated induction of pro-**
115 **inflammatory responses.**

116 To investigate if SARS-CoV-2 spike protein induces production of pro-inflammatory
117 cytokines, we used a minimally immortalised human bronchial epithelial cell line BCI-NS1.1,
118 which was derived from human primary bronchial epithelial cells (Hayman et al., 2019; Hu et
119 al., 2019; Kedzierski et al., 2017; Walters et al., 2013). BCI-NS1.1 was stimulated with
120 histidine (His)-tagged CoV2-S1, CoV2-RBD, or CoV-S1 or CoV-RBD. Both S1 and RBD of
121 CoV2 induced robust and similar levels of pro-inflammatory cytokines (IL-6, IL-1 β , and
122 TNF α) in a dose-dependent manner at 24 hours (hrs) post stimulation (10 – 100ng; Figure 1A
123 – C). The inductions of these pro-inflammatory cytokines by CoV2-S1 were significantly
124 greater than that induced by CoV-S1 (50ng/mL; Figure 1D – F). Higher cytokine inductions
125 by CoV2-S1 were associated with earlier (6hr) and higher NF- κ B activation (phospho (p)-
126 p65) compared to that induced by CoV-S1 (Figure 1G).

127

128 **CoV2-S1-mediated NF- κ B activation is dependent on S1-ACE2 interaction.**

129 Immunoprecipitation using anti-His antibody demonstrates that CoV2-S1 / -RBD binding to
130 ACE2 30 minutes post stimulation (Figure 2A). To further assess if CoV2-S1 and ACE2
131 interaction is required for NF- κ B activation, we have designed six antagonistic peptides of
132 varying lengths (AP-1 – 6; 8 – 15 amino acid residues) that were N-terminally biotinylated
133 (Figure 2B). These peptides were designed based on the contact residues on ACE2 in an
134 attempt to inhibit CoV2-S1-ACE2 interaction, including Y449, Y453, L455, F486, N487,
135 Y489, Q493, Q498, T500, N501, G502, and Y505 on CoV2-S1 (Figure 2B; Table 1) (Li et
136 al., 2005; Wang et al., 2020a).

137

138 We first screened for abilities of these peptides in reducing p65 phosphorylation by CoV2-
139 S1.

140 Treatment of BCI-NS1.1 with AP-6, but not AP-1 – 5, reduced p65 phosphorylation levels
141 induced by CoV2-S1 (Figure 2c; 10 μ M). AP-6 mediated reduction in p65 phosphorylation
142 occurred in a dose-dependent manner (10 – 25 μ M) with minimal cell death (Figure S1A).
143 Higher dose (50 μ M) resulted in increased cell death (Figure S1A). We then assessed if AP-6
144 inhibits S1-ACE2 interaction. In the AP-6 treated group, immunoprecipitation using anti-His
145 antibody also shows reduced CoV2-S1 interaction with ACE2 compared with CoV2-S1-
146 stimulated and non-AP-6-treated group (U; Figure 2E). Conversely, immunoprecipitation

147 using streptavidin diminished interactions between CoV2-S1 and ACE2 in AP-6 group and
148 not in the non-AP-6-treated group. AP-6 also reduced CoV-S1 and ACE2 interaction,
149 indicating the inhibition potentials of AP-6 across small differences in amino acid residue
150 sequences between CoV2-S1 and CoV-S1 RBM (Figure S1B – C).

151

152 This indicates that CoV2-S1 elicits higher inflammatory responses than CoV-S1, and that the
153 CoV2-RBD alone is sufficient for inducing this response. CoV2-S1-ACE2 interaction is
154 required for this heightened inflammatory response.

155

156 **CoV2-S1-ACE2 facilitates NF- κ B activation via MAP kinase signalling.**

157 We investigated if S1-mediated production of pro-inflammatory cytokines requires common
158 adaptor proteins MyD88 or TRIF. Knockdown of MyD88 or TRIF inhibited S1-mediated p65
159 phosphorylation (Figure 3A), indicating MyD88 and TRIF as important signalling adaptors in
160 S1-driven p65 activation. However, immunoprecipitation using anti-His antibody did not pull
161 down MyD88 or TRIF (Figure 3B) 2hrs post stimulation. This indicates an intermediate
162 signalling factor is involved between ACE2 and MyD88/TRIF.

163

164 MyD88 and TRIF activates multiple pathways that converge at NF- κ B. To determine how
165 CoV2-S1 up-regulates NF- κ B activity, we used RT² ProfilerTM Human Inflammasome PCR
166 array and investigated intracellular signalling pathways involved in NF- κ B activation (Figure
167 3C). CoV2-S1 stimulation in BCI-NS1.1 increased expression of genes involved in NF- κ B
168 (*NFKB1*, *NFKBIA*, *NFKBIB*, *RELA*, *TAB1*) and inflammasome signalling (*NLRP1/3/4/6*,
169 *AIM2*, *CASP1*, *PYCARD*, *IL1B*, and *IL18*), but also several factors involved in MAP kinase
170 pathway (*MAPK1/3/3K7/8/9/11/12/13*) (Figure 3D – F; Figure S2).

171

172 MAP kinase pathways have been shown to be involved in NF- κ B activations (Bergmann et
173 al., 1998; Madrid et al., 2001; Wang et al., 2019; Wesselborg et al., 1997), and consistent
174 with the PCR array. Here CoV2-S1 stimulation in BCI-NS1.1 cells also induced heightened
175 activation of MAP kinase p38 (12 – 24hrs), Erk (2 and 24hrs), and Jnk (24hrs), compared to
176 inductions by CoV-S1 (Figure 4A). Knockdown of p38, Erk or Jnk expressions by siRNAs
177 all resulted in a significant reduction in phosphorylation of p65 following CoV2-S1 treatment
178 (Figure 4B), indicating that the MAP kinase is an important contributor to CoV2-S1-
179 mediated NF- κ B activation.

180

181 **CoV2-S1 stimulates rapid ER stress and UPR that activate NF- κ B *via* UPR-MAP kinase**
182 **crosstalk.**

183 CoV2-S1 induced early activation of Erk, which is a kinase utilised by not only MAP kinase
184 but also ER stress and UPR. ER stress has been shown to be induced by the CoV spike
185 protein (Versteeg et al., 2007). ER-UPR features three main pathways, PERK-Erk-CHOP
186 pathway that modulates apoptosis, ATF6 that regulates protein folding, and the IRE1 α –
187 TRAF2 pathway that promotes NF- κ B and p38 activation (Pathinayake et al., 2018).

188 CoV2-S1 and -RBD resulted in an early increase of IRE1 α and PERK activations at 2 and
189 6hrs, respectively, and this was sustained to 24hr post stimulation. Furthermore, IRE1 α and
190 PERK activations were higher compared with CoV-S1 and -RBD (Figure 5A). ATF6
191 activation was equivalent for CoV2-S1 and CoV-S1. As IRE1 α activation was induced
192 earlier (2hrs) by CoV2-S1 than MAP kinases, we then investigated if increased ER-UPR by
193 CoV2-S1 leads to heightened MAP kinase activities by siRNAs.

194

195 Knockdown of PERK resulted in reduced phosphorylation levels of Erk, but had minimal
196 effect on p65, p38, and Jnk activation (Figure 5B). In contrast, reduction of IRE1 α
197 expression decreased p65, p38, and Erk phosphorylation/expression. This indicates that
198 IRE1 α , and not PERK and ATF6 pathway, is involved in p65 activation.

199 We also assessed if MAP kinase modulates ER-UPR. While knockdown of either p38, Erk,
200 or Jnk decreased p65 phosphorylation (Figure 4B), reduction of p38 or Erk gene expression
201 led to decreased PERK phosphorylation but not IRE1 α activation (Figure 5C). Jnk
202 knockdown led to reduced IRE1 α phosphorylation and had no effect on PERK activation.
203 This demonstrates CoV2-S1 promotes NF- κ B activation *via* the ER-UPR (IRE1 α /PERK) and
204 MAP kinase pathway.

205

206 **CoV2-S1 induces markers of acute bronchial epithelial injury**

207 Acute lung injury is a feature observed with severe COVID-19 (Huang et al., 2020; Lai et al.,
208 2017), and markers associated with epithelial damage IL-1 β , TNF α , and CCL2 were all
209 markedly increased by CoV2-S1 in our targeted PCR array (Figure 3C), and at protein levels
210 (Figure 1A). CoV2-S1 also significantly up-regulated protein productions of CCL2 in BCi-
211 NS 1.1 cells (Figure 6A). To further investigate if CoV2-S1 induces epithelial damage, we
212 stimulated differentiated primary bronchial epithelial cells (pBECs) cultured at air-liquid
213 interface (ALI) with CoV2-S1 (50 and 100ng). Stimulation resulted in a significant reduction

214 in transepithelial electrical resistance (TEER; Figure 6B), demonstrating an increased
215 epithelial permeability and tight junction disruption caused by CoV2-S1. This is
216 accompanied with increased protein production of epithelial damage factors IL-1 β , TNF α ,
217 and CCL2, in a CoV2-S1 dose-dependent manner (Figure 6C – E).

218 Immunofluorescent staining of a tight junction protein zonula occludens-1 (ZO-1) showed
219 strong localisation at the cell borders in the non-stimulated controls, whereas CoV2-S1 led to
220 partial disappearance of ZO-1 at the cell borders (Figure 6F). This indicates that CoV2-S1
221 may also disrupt epithelial barrier function.

222

223 **CoV2-S1-antagonistic AP-6 and FDA-approved ER stress and MAP kinase inhibitors** 224 **ameliorate CoV2-mediated inflammatory response.**

225 CoV2-S1 induced NF- κ B activation and subsequent production of pro-inflammatory
226 cytokines was dependent on ACE2 interaction and early ER-UPR and MAP kinase activities.
227 We therefore assessed whether CoV2-S1-mediated inflammation could be reduced by our
228 CoV2-S1 inhibitory peptide AP-6, or with existing FDA-approved pharmacological
229 inhibitors, that target ER stress (4-PBA) or MAP kinase (trametinib and ulixertinib).

230

231 Treatment with AP-6 led to a significant decrease in CoV2-S1-mediated phosphorylation of
232 p65 (Figure 2C) and production of IL-6, IL-1 β , TNF α , but not CCL2 (Figure 7A – D) in a
233 dose-dependent manner. Similarly, the ER stress inhibitor 4-PBA and both MAP kinase
234 inhibitors led to decreased activation of p65 (Figure S3) and expression of these pro-
235 inflammatory and epithelial injury cytokines (Figure 7E – L). These drugs had no effect on
236 non-CoV2-S1-stimulated controls (Figure S4). CoV2-S1-induced reduction in barrier
237 integrity and ZO-1 was prevented *via* treatment with AP-6 and FDA-approved inhibitors,
238 suggesting maintained barrier function (Figure S5A – B). This strongly indicates that AP-6
239 could serve as a proof-of-concept therapeutic peptides against SARS-CoV-2 and also that ER
240 stress / MAP kinase inhibitors could be used to reduce inflammation and lung injury caused
241 by CoV2-S1.

242

243 **Discussion**

244 CoV Spike protein and host ACE2 interaction is a critical first step to viral replication and
245 diseases. Here we demonstrate that CoV2 S1 subunit and RBD induces early ER-UPR and
246 MAP kinase activations, leading to hyper-inflammatory responses. Our results indicate that

247 this inflammatory storm, and downstream consequences are inhibited by S1-inhibitory
248 peptides and existing FDA-approved ER stress and MAP kinase inhibitors (Figure 8).

249

250 COVID-19 has been shown to be associated with increased plasma levels of pro-
251 inflammatory cytokines including IL-6 and TNF α (Huang et al., 2020; McGonagle et al.,
252 2020; Mehta et al., 2020). SARS-CoV-2 S1 and RBD alone induced heightened levels of IL-
253 6, TNF α and IL-1 β , and the productions of which were higher compared with CoV-S1. This
254 induction occurs in an ACE2-dependent manner, and the higher affinity of CoV2-S1 towards
255 ACE2 may have contributed to this elevated inflammatory response (Wang et al., 2020a).
256 CoV2-S1-mediated NF- κ B activation is also dependent on common TLR adaptor proteins
257 MyD88 and TRIF, although we could not detect interactions between ACE2 and these
258 adaptor proteins. It is possible that CoV2-S1-ACE2 interaction triggers MyD88 and TRIF
259 activations *via* other signalling factors that then result in NF- κ B activation.

260

261 Our data strong indicates that inflammation could be triggered by CoV2-S1 even before viral
262 replication occurs or in the absence of viral replication. As CoV2-S1 is present throughout
263 viral replication cycles and infection, our data demonstrate that spike proteins are likely to be
264 a major contributor to inflammation. The constant presence of S1 is consistent with the high
265 viral load and a long virus-shedding period observed in patients with severe COVID-19 (Liu
266 et al., 2020). Furthermore, CoV2 spike protein has an estimated half-life of 30 hours in
267 mammalian systems (*in silico* analysis by ExPASy ProtParam) and consists of amino acid
268 residues with long half-lives (leucine (8%), threonine (7.6%), valine (7.6%), alanine (6.2%),
269 glycine (6.4%), and proline (4.6%)). This may suggest persistent presence of CoV2 spike
270 protein from live and dead viruses that plays major role in triggering the inflammatory storm
271 in COVID-19. Importantly, our antagonistic peptide inhibited this binding event and reduced
272 the production of pro-inflammatory cytokines. While peptide sequence optimisations are
273 required to further increase effectiveness and stability, this highlights the potentials of using
274 S1 antagonistic peptides as neutralising molecules against SARS-CoV-2.

275

276 The ER is mainly involved in protein folding, trafficking and post-translational modification
277 of secreted and transmembrane proteins (Pathinayake et al., 2018). Viral infections and
278 inflammatory cytokines result in high ratio of misfolded or unfolded proteins in the ER,
279 leading to UPR (Osowski and Urano, 2011). This adaptive mechanism triggers a range of
280 different innate immune responses *via* different UPR pathways; PERK-Erk-CHOP pathway

281 that halts protein translation (Harding et al., 2000); ATF6 that facilitates protein folding as
282 well as NF- κ B activation (Shen et al., 2005); and IRE1 \square promotes NF- κ B phosphorylation
283 and inflammation (Urano et al., 2000). Here we showed that both ER-UPR and MAP kinases
284 modulate CoV2-S1-induced NF- κ B activations.

285

286 CoV2-S1 caused early activation of ER-UPR IRE1 \square and PERK-Erk, leading to MAP kinases
287 phosphorylations, which then facilitated NF- κ B-mediated production of pro-inflammatory
288 cytokines. Although IRE1 \square activation occurred earlier than other ER-UPR and MAP kinase
289 factors, it is likely that CoV2-S1 can promote NF- κ B activation *via* ER-UPR and MAP
290 kinase both independently and cooperatively (Bergmann et al., 1998; Madrid et al., 2001;
291 Wesselborg et al., 1997) (Harding et al., 2000; Urano et al., 2000). Our result is consistent
292 with previous reports that showed ER stress and MAP kinase activation by SARS-CoV
293 infection (Lee et al., 2004; Mizutani et al., 2004; Versteeg et al., 2007), we cannot rule out
294 the possibilities of other mechanisms of ER-UPR and MAP kinase activations by CoV2-S1.
295 Furthermore, during SARS-CoV-2 infection, viral RNAs will also trigger inflammatory
296 responses *via* multiple pattern recognition receptors including TLRs and RLRs. This together
297 with spike proteins may instigate more exaggerated inflammatory responses during SARS-
298 CoV-2 infection.

299

300 Acute lung injury is a feature of severe COVID-19 (Huang et al., 2020; McGonagle et al.,
301 2020; Mehta et al., 2020). Surprisingly we found that CoV2-S1 and -RBD alone was also
302 sufficient to reduce epithelial barrier function through the release of IL-1 β , TNF \square , and
303 CCL2. Pro-inflammatory cytokines IL-1 β and TNF \square have been shown to induce epithelial
304 damage by further promoting p38 and NF- κ B activation (Al-Sadi et al., 2013; Kimura et al.,
305 2013; Kimura et al., 2009; Kolb et al., 2001; Sheridan et al., 1997). CCL2 is a chemokine that
306 has been shown to be transcriptionally driven by NF- κ B and is typically released by injured
307 tissues and attracts macrophages to the site of infection and inflammation (Kavandi et al.,
308 2012; Lai et al., 2017).

309 While CoV2-S1 increased membrane permeability that was consistent with small loss of ZO-
310 1 localisation at cell-cell junctions, it is likely that increased production of these
311 inflammatory and injury-related factors from epithelial cells recruit macrophages to the site
312 of infection, which then promote excessive tissue damage. These inflammatory and injury-
313 stimulated factors as well as macrophages have been reported to be highly elevated in those

314 with severe COVID-19 (Huang et al., 2020; McGonagle et al., 2020; Mehta et al., 2020),
315 further indicating that this “inflammatory injury” can be driven by CoV2-S1 in severe
316 COVID-19, and may be the first step to ARDS.

317

318 Increased ER-UPR and MAP kinase activities as well as pro-inflammatory responses induced
319 by CoV2-S1 could be substantially reduced by FDA-approved ER stress inhibitor 4-PBA and
320 MAP kinase inhibitors trametinib and ulixertinib. 4-PBA is a chemical chaperone currently
321 used for treatment of urea cycle disorder (Lichter-Konecki et al., 2011), and has been used in
322 clinical trials for diabetes (Ozcan et al., 2006), cystic fibrosis (Ozcan et al., 2006), sickle cell
323 disease (Collins et al., 1995), and neurodegenerative diseases (Mimori et al., 2012).
324 Trametinib is a MAP kinase inhibitor used for melanoma (Hoffner and Benchich, 2018), and
325 ulixertinib is a highly potent, selective, reversible, ERK1/2 inhibitor used as cancer treatment
326 (Sullivan et al., 2018). Re-purposing these drugs that has a well-documented safety profile in
327 humans could expedite rapid deployment of these drugs for severe COVID-19.

328

329 Collectively, CoV2-S1 induced heightened production of inflammatory cytokines that are
330 primarily driven by MAP kinase and ER stress cross-talks. CoV2-S1 AP-6 demonstrates the
331 feasibility of this proof-of-concept CoV-specific antiviral strategy. While antiviral drugs and
332 vaccines are being developed and assessed, existing FDA-approved ER stress and MAP
333 kinase inhibitors could be immediately deployed in clinical trials as a potential treatment
334 options for those with severe COVID-19.

335

336 **Acknowledgments**

337 This study is funded by University of Newcastle Faculty Pilot Grant (1032380) and John
338 Hunter Hospital Charitable Trust (G1700465).

339 The authors have declared that no conflict of interest exists.

340

341 **Author Contributions**

342 Conceptualization, A. C-Y. H.; Methodology, A. C-Y. H., G. W., A. T. R., P. C. V., P. S.P.;
343 Investigation, A. C-Y. H., G. W., K. D., A. T. R., P. C. V.; Validation, G. W., A. T. R., P. C.
344 V., P. S.P., K. D.; Writing – Original and finalised version, A. C-Y. H.; Writing – Review &
345 Editing, A. C-Y. H., G. W., A. T. R., P. C. V., P. S.P., K. D., J. C. H., F. W., P. A. W.;
346 Funding Acquisition, A. C-Y. H.; F. W.

347

348 **Declaration of Interests**

349 The authors declare no competing interests.

350

351 **Figure legends**

352 **Figure 1. CoV2-S1 and -RBD stimulated higher productions of IL-6, IL-1 β and TNF α**
353 **compared with CoV-S1 and -RBD.**

354 **A-C.** Protein levels of IL-6, IL-1 β and TNF α stimulated by SARS-CoV-2 spike subunit 1
355 (CoV2-S1) and receptor binding domain (RBD) 24hrs post stimulation. **D-F.** Stimulation and
356 comparison of IL-6, IL-1 β and TNF α by CoV2-S1 and CoV-S1. **G.** Immunoblot (left) and
357 densitometry (right) of induction kinetics of phospho(p)-p65 at 2, 6, 12, and 24hr post
358 stimulation by CoV2-S1 and CoV-S1 compared with CoV2-S1 stimulated but untreated
359 control (U). N = 6 showing mean \pm SEM. Immunoblots shown are representative of three
360 independent experiments and β -actin was detected to show equal protein input (lower panel).
361 * $P \leq 0.05$ versus untreated control.

362

363 **Figure 2. CoV2-S1-mediated NF- κ B activation requires ACE2 interaction and can be**
364 **inhibited by antagonistic peptides.**

365 **A.** Immunoblot of ACE2 after immunoprecipitation using anti-His antibody 30 minutes post
366 CoV2-S1/-RBD or CoV-2-S1/-RBD stimulation. **B.** Schematic representation of CoV2-S1
367 protein and peptide design coverage. **C.** Immunoblot of p-p65 and p65 24hrs post CoV2-S1
368 stimulation and CoV2-S1 antagonistic peptides (AP-1 – 6) compared with untreated control
369 (U). **D.** Immunoblot (left) and densitometry (right) of p-p65 and p65 at 24hrs post CoV2-S1
370 and AP-6 (10, 25 μ M) treatment compared with CoV2-S1 stimulated but untreated control
371 (U). **E.** Immunoblot of ACE2 and His following immunoprecipitation using anti-His antibody
372 30 minutes post CoV2-S1/AP-6 treatment. Immunoblot of His and ACE2 following
373 immunoprecipitation using streptavidin-coupled Dynabeads 30 minutes post CoV2-S1/AP-6
374 treatment. N = 3 showing mean \pm SEM. Immunoblots shown are representative of three
375 independent experiments and β -actin was detected to show equal protein input (lower panel).
376 * $P \leq 0.05$ versus untreated control.

377

378 **Figure 3. CoV2-S1 stimulates NF- κ B activation via MyD88/TRIF adaptors and MAP**
379 **kinase pathway.**

380 **A.** Immunoblots of p-p65 phosphorylation 24hrs post CoV2-S1 stimulation in MyD88 and
381 TRIF silenced BCi-NS1.1. **B.** Immunoblots of MyD88 and TRIF after immunoprecipitation
382 using anti-His antibody 2 hours post stimulation. **C-F.** CoV2-S1-stimulated BCi-NS1.1 cells
383 (24hrs) were subjected to RT² ProfilerTM Human Inflammasone PCR array with highly up-
384 regulated genes involved in NF- κ B, Inflammasome, and MAP kinase pathways. N = 3 for
385 Figure 3A-B. Immunoblots shown are representative of three independent experiments and β -
386 actin was detected to show equal protein input (lower panel).

387

388 **Figure 4. CoV2-S1 mediates NF- κ B activation via MAP kinase pathways.**

389 **A.** Immunoblot (top) and densitometry (bottom) of induction kinetics of p-p38, p38, p-Erk,
390 Erk, p-Jnk and Jnk at 2, 6, 12, and 24hr post stimulation by CoV2-S1 and CoV-S1 compared
391 with untreated control (U). **B.** Immunoblots of p-p65, p65, p-p38, p38, p-Erk, Erk, p-Jnk and
392 Jnk 24hrs post CoV2-S1 or -RBD stimulation in p38, Erk or Jnk silenced BCi-NS1.1. N = 3.
393 Immunoblots shown are representative of three independent experiments and β -actin was
394 detected to show equal protein input (lower panel). * $P \leq 0.05$ versus untreated control.

395

396 **Figure 5. CoV2-S1 induces rapid ER-UPR that activate NF- κ B via ER-MAP kinase**
397 **crosstalk.**

398 **A.** Immunoblot (top) and densitometry (bottom) of induction kinetics of p-IRE1 α , IRE1 α , p-
399 ATF6, ATF6, p-PERK and PERK at 2, 6, 12, and 24hr post stimulation by CoV2-S1 and
400 CoV-S1 compared with untreated control (U). **B.** Immunoblots of p-p65, p65, p-p38, p38, p-
401 Erk, Erk, p-Jnk, Jnk, IRE1 α and PERK 24hrs post CoV2-S1 or -RBD stimulation in PERK
402 and IRE1 α silenced BCi-NS1.1. **C.** Immunoblots of p-PERK and p-IRE1 α post CoV2-S1 or
403 -RBD stimulation in p38, Erk or Jnk silenced BCi-NS1.1. N = 3. Immunoblots shown are
404 representative of three independent experiments and β -actin was detected to show equal
405 protein input (lower panel). * $P \leq 0.05$ versus untreated control.

406

407 **Figure 6. CoV2-S1 promotes epithelial damage.**

408 **A.** CCL2 protein production at 24hrs post CoV2-S1 stimulation in BCi-NS1.1. **B.** Percentage
409 changes in transepithelial electrical resistance (TEER) in differentiated primary bronchial
410 epithelial cells (pBECs) cultured at air-liquid interface (ALI) 24hrs post CoV2-S1
411 stimulation. **C-E.** Protein levels of IL-1 β , TNF α , and CCL2 at 24hrs post CoV2-S1

412 stimulation in pBECs-ALI. N = 3. * $P \leq 0.05$ versus untreated control. **F.** Immunofluorescent
413 images of ZO-1 labelling. N = 2.

414

415 **Figure 7. CoV2-S1 antagonistic peptide AP-6 and FDA-approved ER stress and MAP**
416 **kinase inhibitors suppress CoV2-S1-mediated production of pro-inflammatory**
417 **cytokines.**

418 **A-D.** Protein levels of IL-6, IL-1 β and TNF α at 24hrs post stimulation by CoV2-S1 and
419 treatment with antagonistic peptide AP-6, **E-H.** 4-PBA, and **I-L.** Trametinib, and Ulixertinib.
420 N = 3. * $P \leq 0.05$ versus untreated control.

421

422 **Figure 8. Schematics of CoV2-S1-mediated inflammation via ER-UPR and MAP kinase**
423 **pathways.**

424 SARS-CoV-2 (CoV2) spike protein binds with ACE2 on the surface of human bronchial
425 epithelial cells and rapidly facilitates the induction of ER stress and unfolded protein
426 response (UPR). Activation of UPR (PERK and IRE1 α) promotes the activation of MAP
427 kinases, and the two pathways synergistically drive the activation of NF- κ B and production
428 of pro-inflammatory cytokines. FDA-approved ER-UPR inhibitor 4-phenylbutyric acid (4-
429 PBA) and MAP kinase inhibitors (trametinib and ulixertinib) suppressed CoV2-S1-induced
430 ER stress and MAP kinase activities, resulting in reduced NF- κ B-mediated expression of pro-
431 inflammatory cytokines.

432

433 **STAR Methods**

434 **Experimental model and subject details**

435 Cell line

436 BCi-NS1.1 cells were obtained from Prof. Ronald Crystal Laboratory at Weill Cornell
437 Medical College, and Memorial Sloan-Kettering Cancer Center, New York, NY, USA)
438 (Walters et al., 2013). The cells were cultured in hormone supplemented Bronchial Epithelial
439 Cell Growth Media (BEGM; Lonza, Switzerland) supplemented with 50U/mL penicillin and
440 streptomycin (Hayman et al., 2019; Hu et al., 2019; Kedzierski et al., 2017).

441

442 Human subject recruitment for pBECs

443 Five healthy control subjects were recruited for bronchoscopy. Healthy non-smoking controls
444 with no evidence of airflow obstruction, bronchial hyper-responsiveness to hypertonic saline
445 challenge, or chronic respiratory symptoms were also recruited. Clinical history, examination

446 and spirometry were performed on all individuals, whom were also questioned about the
447 previous severity of cold symptoms. At the time of recruitment none of the subjects had
448 symptoms of acute respiratory tract infections for the preceding four weeks and did not have
449 a diagnosis of lung cancer.

450 All subjects gave written informed consent. All procedures were performed according to
451 approval from The University of Newcastle Human Ethics Committees

452

453 Differentiation of primary bronchial epithelial cells (pBECs) at air-liquid-interface (ALI)

454 Human pBECs were obtained by endobronchial brushing and research bronchoscopy in
455 accordance with standard guidelines. pBECs were cultured in BEGM in polycarbonate tissue
456 culture flasks as previously described (Hsu et al., 2011; Hsu et al., 2017; Hsu et al., 2012;
457 Hsu et al., 2016; Hsu et al., 2015; Kedzierski et al., 2017; Parsons et al., 2014; Vanders et al.,
458 2019), and were then cultured on polyester membrane transwells (12mm diameter, 0.4µM
459 pore size, Corning, USA) under submerged condition in ALI initial media (31.25mL low
460 glucose DMEM and BEGM, 1µL of 1mM All-trans retinoic acid, 4µL of 25µg/mL
461 recombinant human epidermal growth factor (rhEGF), 62.5µL hydrocortisone, bovine
462 insulin, epinephrine, transferrin, 80µM ethanolamine, 0.3mM MgCl₂, 0.4mM MgSO₄,
463 0.5mg/mL BSA, and 250µL bovine pituitary extract, supplemented with
464 penicillin/streptomycin and amphotericin B). When cells become fully confluent, pBECs
465 were air-lifted by removing apical media and changing basal media into ALI final media (as
466 described above for ALI initial media but with 0.5ng/mL of rhEGF). Transepithelial
467 resistance (TEER) was measured every seven days using a EVOM2 Epithelial Voltohmmeter
468 (World Precision Instruments, USA). The basal media was replaced with fresh ALI final
469 media every second day. pBECs were cultured at ALI for 25 days and then used for
470 experiments. All cells were cultured and maintained at 37°C / 5% CO₂.

471

472 **Method details**

473 Spike protein subunit 1 (S1) and receptor binding domain (RBD) stimulation

474 For cells cultured in submerged and at ALI conditions, his-tagged S1 and RBD (Sino
475 Biological Inc.) was diluted in BEBM minimal media (Lonza, Switzerland) and added to the
476 cells. For pBECs at ALI, S1 and RBD was added to the apical side.

477

478 CoV2-S1 antagonistic peptides

479 Six peptides of varying lengths (8 – 15 amino acid residues) were designed based on the
480 amino acid residues critical in ACE2 binding within the receptor binding motif (RBM) of the
481 receptor binding domain (RBD). The peptides were biotinylated at the amino-terminus with
482 carboxy-terminal amidation. The peptides were synthesised by GenScript Biotech Corp. The
483 peptides were added to the BCI-NS1.1 at 10 or 25µM, or added to pBECs-ALI (25µM) at the
484 apical side.

485

486 Drugs

487 ER stress inhibitor 4-phenylbutyric acid (4-PBA) was purchased from Sigma-Aldrich, and
488 resuspended in H₂O and diluted in culture media. MAP kinase inhibitors trametinib
489 (GSK1120212) and ulixertinib (BVD-523) were purchased from Selleck Chemicals and were
490 resuspended and diluted in DMSO.

491

492 Cell viability

493 Cell viability was measured using PE Annexin V Apoptosis Detection kit I (Becton
494 Dickinson) according to manufacturer's instruction. Cells were stained with annexin V-PE
495 and vital dye 7-amino-actinomycin (7-AAD), and then analyzed using a FACSCanto II
496 (Becton Dickinson) and FACSDiva software. Viable cells were stained AxV negative / 7-
497 AAD negative and expressed as percentage of total analyzed cells.

498

499 siRNAs

500 siRNAs specific to MyD88, TRIF, p38, Erk, Jnk, PERK, and IRE1 α (Life Technologies,
501 USA) were reverse transfected into cells using siPORT NeoFX transfection agent (Ambion,
502 USA) according to manufacturer's instruction. Silence Negative controls (Life Technologies,
503 USA) were used as siRNA negative controls.

504

505 PCR array

506 RNAs from S1-stimulated cells were extracted using RNeasy Mini Kits and QIAcube
507 (Qiagen, USA) according to manufacturer's instruction. 200ng of RNAs were reverse
508 transcribed to cDNA using random primers (Applied Biosystem, USA). cDNAs were then
509 subjected to pathway-focused gene expression array using RT² ProfilerTM Human
510 Inflammasome PCR array (Qiagen, USA). The raw data was analysed by the Data Analysis
511 Center on Qiagen website ([https://www.qiagen.com/mx/shop/genes-and-pathways/data-
512 analysis-center-overview-page/](https://www.qiagen.com/mx/shop/genes-and-pathways/data-analysis-center-overview-page/)).

513

514 Immunoblotting and immunoprecipitation

515 S1-stimulated cells were lysed in protease-inhibitor cocktail supplemented RIPA buffer
516 (Roche, UK). Proteins were subjected to SDS-PAGE (Bio-Rad Laboratories, USA) and
517 transferred onto polyvinylidene fluoride membranes (Merck-Milipore, USA). Proteins were
518 detected using antibodies to His, MyD88, TRIF, p65, phospho-(p)-p65, p38, p-p38, Erk, p-
519 Erk, Jnk, p-Jnk, PERK, p-PERK, IRE1 α , and p-IRE1 α antibodies (All from Abcam, UK).
520 Antibody to ACE2 was obtained from RnD Systems (USA). For immunoprecipitation, whole
521 cell lysates (1mg) were immunoprecipitated using anti-His antibody or isotype antibody
522 (Abcam, UK), streptavidin-coupled DynabeadsTM M-280 and DynabeadsTM His-tag isolation
523 & Pulldown kit (Life Technologies, USA) according to manufacturer's instruction. Proteins
524 were detected using SuperSignal WestFemto Maximum Sensitivity Substrate (Thermo Fisher
525 Scientific, USA) and visualised on a ChemiDoc MP Imaging system (Bio-Rad Laboratories,
526 USA).

527

528 Immunofluorescent microscopy

529 Treated pBECs cultured at ALI were fixed 4% paraformaldehyde and blocked with 50mM
530 glycine overnight, and then stained with anti-ZO-1 antibody (Thermo Fisher Scientific, USA)
531 or rabbit isotype IgG (Abcam, UK), counter stained with DAPI (Life Technologies, USA),
532 and viewed under a Axio Imager M2 microscope and analyzed using Zen imaging software
533 (Zeiss) as described previously (Liu et al., 2019; Liu et al., 2016; Liu et al., 2017; Reid et al.,
534 2020).

535

536 Cytometric bead array and ELISA

537 Human IL-6, IL-1 β , and TNF α concentrations were determined by cytometric bead array and
538 flow cytometry (FACSCanto II flow cytometer; BD Biosciences, USA) according to the
539 manufacturer's instructions. Human CCL2 and TGF β was measured by ELISA according to
540 the manufacturer's instructions (R&D Systems, USA).

541

542 **Quantification and statistical analysis**

543 Statistical analysis

544 Data were analysed on GraphPad Prism 8. Statistical significance of differences was assessed
545 using parametric Student's two tailed t test for normally distributed data and Mann-Whitney
546 U test for non-parametric data. Differences were considered significant when $p < 0.05$.

547

548 **Figure S1. AP-6 reduces CoV-S1 and ACE2 interaction.**

549 **A.** Cell viability measured by annexin-V/7-AAD staining and flow cytometry. **B.**
550 Immunoblot of ACE2 and His following immunoprecipitation using anti-His antibody 30
551 minutes post CoV2-S1/AP-6 treatment. N = 3. Immunoblots shown are representative of
552 three independent experiments. **C.** Amino acid residue sequence alignments of CoV2-S1 and
553 CoV-S1. Blue = AP-6 contact region. Sequence alignment performed by Clustal Omega.

554

555 **Figure S2. CoV2-S1 up-regulates genes involved in inflammation, inflammasome and**
556 **MAP kinase pathways.**

557 CoV2-S1-stimulated BCi-NS1.1 cells were subjected to RT² ProfilerTM Human
558 Inflammasome PCR array with highly up-regulated genes involved in NF- κ B, Inflammasome,
559 and MAP kinase pathways. N=3.

560

561 **Figure S3. CoV2-S1 antagonistic peptides, Trametinib, and Ulixertinib had no effect on**
562 **IL-6, IL-1 β , TNF α , and CCL2 productions in non-CoV2-S1-treated cells.**

563 Protein levels of IL-6, IL-1 β , TNF α , and CCL2 induced by **A-D** antagonistic peptide (AP-6),
564 **E-H.** 4-PBA, **I-L.** trametinib, and **M-P.** ulixertinib. N = 3.

565

566 **Figure S4. FDA-approved ER stress and MAP kinase inhibitors suppress CoV2-S1-**
567 **mediated NF- κ B activation.**

568 Immunoblot (top) and densitometry (bottom) of p-p65 and p65 at 24hr post stimulation by
569 CoV2-S1 or -RBD compared with vehicle control. Immunoblots shown are representative of
570 three independent experiments and β -actin was detected to show equal protein input (lower
571 panel).

572

573 **Figure S5. AP-6, 4-PBA, trametinib and ulixertinib improves epithelial permeability.**

574 **A.** Percentage changes in transepithelial electrical resistance (TEER) in differentiated
575 primary bronchial epithelial cells (pBECs) cultured at air-liquid interface (ALI) at 24hrs post
576 CoV2-S1 stimulation and treatment with either AP-6, 4-PBA, trametinib, and ulixertinib. N =
577 3. * $P \leq 0.05$ versus non-CoV2-S1-stimulated control, + versus CoV2-S1-stimulated group. **B.**
578 Immunofluorescent images of ZO-1 staining. N = 2.

579

580

581 **References**

- 582 Al-Sadi, R., Guo, S., Ye, D., Dokladny, K., Alhmoud, T., Ereifej, L., Said, H.M., and Ma, T.Y.
583 (2013). Mechanism of IL-1beta modulation of intestinal epithelial barrier involves p38 kinase and
584 activating transcription factor-2 activation. *J Immunol* 190, 6596-6606
- 585 Alexopoulou, L., Holt, A.C., Medzhitov, R., and Flavell, R.A. (2001). Recognition of double-stranded
586 RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 413, 732-738
- 587 Bergmann, M., Hart, L., Lindsay, M., Barnes, P.J., and Newton, R. (1998). IkappaBalpha degradation
588 and nuclear factor-kappaB DNA binding are insufficient for interleukin-1beta and tumor necrosis
589 factor-alpha-induced kappaB-dependent transcription. Requirement for an additional activation
590 pathway. *The Journal of biological chemistry* 273, 6607-6610
- 591 Collins, A.F., Pearson, H.A., Giardina, P., McDonagh, K.T., Brusilow, S.W., and Dover, G.J. (1995).
592 Oral sodium phenylbutyrate therapy in homozygous beta thalassemia: a clinical trial. *Blood* 85, 43-49
- 593 Diebold, S.S., Kaisho, T., Hemmi, H., Akira, S., and Reis e Sousa, C. (2004). Innate antiviral
594 responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 303, 1529-1531
- 595 Guan, X., Yuan, Y., Wang, G., Zheng, R., Zhang, J., Dong, B., Ran, N., Hsu, A.C., Wang, C., and
596 Wang, F. (2020). Ginsenoside Rg3 ameliorates acute exacerbation of COPD by suppressing
597 neutrophil migration. *Int Immunopharmacol* 83, 106449
- 598 Harding, H.P., Zhang, Y., Bertolotti, A., Zeng, H., and Ron, D. (2000). Perk is essential for
599 translational regulation and cell survival during the unfolded protein response. *Molecular cell* 5, 897-
600 904
- 601 Hayman, T.J., Hsu, A.C., Kolesnik, T.B., Dagley, L.F., Willemsen, J., Tate, M.D., Baker, P.J.,
602 Kershaw, N.J., Kedzierski, L., Webb, A.I., *et al.* (2019). RIPLET, and not TRIM25, is required for
603 endogenous RIG-I-dependent antiviral responses. *Immunology and cell biology* 97, 840-852
- 604 Hoffner, B., and Benchich, K. (2018). Trametinib: A Targeted Therapy in Metastatic Melanoma. *J*
605 *Adv Pract Oncol* 9, 741-745
- 606 Hsu, A.C., Barr, I., Hansbro, P.M., and Wark, P.A. (2011). Human influenza is more effective than
607 avian influenza at antiviral suppression in airway cells. *American journal of respiratory cell and*
608 *molecular biology* 44, 906-913
- 609 Hsu, A.C., Dua, K., Starkey, M.R., Haw, T.J., Nair, P.M., Nichol, K., Zammit, N., Grey, S.T., Baines,
610 K.J., Foster, P.S., *et al.* (2017). MicroRNA-125a and -b inhibit A20 and MAVS to promote
611 inflammation and impair antiviral response in COPD. *JCI Insight* 2, e90443
- 612 Hsu, A.C., Parsons, K., Barr, I., Lowther, S., Middleton, D., Hansbro, P.M., and Wark, P.A. (2012).
613 Critical role of constitutive type I interferon response in bronchial epithelial cell to influenza
614 infection. *PLoS One* 7, e32947
- 615 Hsu, A.C., Parsons, K., Moheimani, F., Knight, D.A., Hansbro, P.M., Fujita, T., and Wark, P.A.
616 (2016). Impaired Antiviral Stress Granule and IFN-beta Enhanceosome Formation Enhances
617 Susceptibility to Influenza Infection in Chronic Obstructive Pulmonary Disease Epithelium. *American*
618 *journal of respiratory cell and molecular biology* 55, 117-127
- 619 Hsu, A.C., Starkey, M.R., Hanish, I., Parsons, K., Haw, T.J., Howland, L.J., Barr, I., Mahony, J.B.,
620 Foster, P.S., Knight, D.A., *et al.* (2015). Targeting PI3K-p110alpha Suppresses Influenza Virus
621 Infection in Chronic Obstructive Pulmonary Disease. *American journal of respiratory and critical care*
622 *medicine* 191, 1012-1023
- 623 Hu, M., Schulze, K.E., Ghildyal, R., Henstridge, D.C., Kolanowski, J.L., New, E.J., Hong, Y., Hsu,
624 A.C., Hansbro, P.M., Wark, P.A., *et al.* (2019). Respiratory syncytial virus co-opts host mitochondrial
625 function to favour infectious virus production. *Elife* 8
- 626 Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., *et al.*
627 (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*
628 395, 497-506
- 629 Kavandi, L., Collier, M.A., Nguyen, H., and Syed, V. (2012). Progesterone and calcitriol attenuate
630 inflammatory cytokines CXCL1 and CXCL2 in ovarian and endometrial cancer cells. *J Cell Biochem*
631 113, 3143-3152
- 632 Kedzierski, L., Tate, M.D., Hsu, A.C., Kolesnik, T.B., Linossi, E.M., Dagley, L., Dong, Z., Freeman,
633 S., Infusini, G., Starkey, M.R., *et al.* (2017). Suppressor of cytokine signaling (SOCS)5 ameliorates
634 influenza infection via inhibition of EGFR signaling. *Elife* 6

635 Kimura, K., Morita, Y., Orita, T., Haruta, J., Takeji, Y., and Sonoda, K.H. (2013). Protection of
636 human corneal epithelial cells from TNF-alpha-induced disruption of barrier function by rebamipide.
637 *Invest Ophthalmol Vis Sci* 54, 2572-2760
638 Kimura, K., Teranishi, S., and Nishida, T. (2009). Interleukin-1beta-induced disruption of barrier
639 function in cultured human corneal epithelial cells. *Invest Ophthalmol Vis Sci* 50, 597-603
640 Kolb, M., Margetts, P.J., Anthony, D.C., Pitossi, F., and Gauldie, J. (2001). Transient expression of
641 IL-1beta induces acute lung injury and chronic repair leading to pulmonary fibrosis. *J Clin Invest* 107,
642 1529-1536
643 Lai, C., Wang, K., Zhao, Z., Zhang, L., Gu, H., Yang, P., and Wang, X. (2017). C-C Motif
644 Chemokine Ligand 2 (CCL2) Mediates Acute Lung Injury Induced by Lethal Influenza H7N9 Virus.
645 *Frontiers in microbiology* 8, 587
646 Le Goffic, R., Pothlichet, J., Vitour, D., Fujita, T., Meurs, E., Chignard, M., and Si-Tahar, M. (2007).
647 Cutting Edge: Influenza A virus activates TLR3-dependent inflammatory and RIG-I-dependent
648 antiviral responses in human lung epithelial cells. *J Immunol* 178, 3368-3372
649 Lee, C.H., Chen, R.F., Liu, J.W., Yeh, W.T., Chang, J.C., Liu, P.M., Eng, H.L., Lin, M.C., and Yang,
650 K.D. (2004). Altered p38 mitogen-activated protein kinase expression in different leukocytes with
651 increment of immunosuppressive mediators in patients with severe acute respiratory syndrome. *J*
652 *Immunol* 172, 7841-7847
653 Li, F., Li, W., Farzan, M., and Harrison, S.C. (2005). Structure of SARS coronavirus spike receptor-
654 binding domain complexed with receptor. *Science* 309, 1864-1868
655 Lichter-Konecki, U., Diaz, G.A., Merritt, J.L., 2nd, Feigenbaum, A., Jomphe, C., Marier, J.F.,
656 Beliveau, M., Mauney, J., Dickinson, K., Martinez, A., *et al.* (2011). Ammonia control in children
657 with urea cycle disorders (UCDs); phase 2 comparison of sodium phenylbutyrate and glycerol
658 phenylbutyrate. *Mol Genet Metab* 103, 323-329
659 Liu, G., Cooley, M.A., Jarnicki, A.G., Borghuis, T., Nair, P.M., Tjin, G., Hsu, A.C., Haw, T.J.,
660 Fricker, M., Harrison, C.L., *et al.* (2019). Fibulin-1c regulates transforming growth factor-beta
661 activation in pulmonary tissue fibrosis. *JCI Insight* 5
662 Liu, G., Cooley, M.A., Jarnicki, A.G., Hsu, A.C., Nair, P.M., Haw, T.J., Fricker, M., Gellatly, S.L.,
663 Kim, R.Y., Inman, M.D., *et al.* (2016). Fibulin-1 regulates the pathogenesis of tissue remodeling in
664 respiratory diseases. *JCI Insight* 1
665 Liu, G., Cooley, M.A., Nair, P.M., Donovan, C., Hsu, A.C., Jarnicki, A.G., Haw, T.J., Hansbro, N.G.,
666 Ge, Q., Brown, A.C., *et al.* (2017). Airway remodelling and inflammation in asthma are dependent on
667 the extracellular matrix protein fibulin-1c. *J Pathol* 243, 510-523
668 Liu, Y., Yan, L.M., Wan, L., Xiang, T.X., Le, A., Liu, J.M., Peiris, M., Poon, L.L.M., and Zhang, W.
669 (2020). Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect Dis*
670 Madrid, L.V., Mayo, M.W., Reuther, J.Y., and Baldwin, A.S., Jr. (2001). Akt stimulates the
671 transactivation potential of the RelA/p65 Subunit of NF-kappa B through utilization of the I kappa B
672 kinase and activation of the mitogen-activated protein kinase p38. *The Journal of biological chemistry*
673 276, 18934-18940
674 Magagnoli, J., Narendran, S., Pereira, F., Cummings, T., Hardin, J.W., Sutton, S.S., and Ambati, J.
675 (2020). Outcomes of hydroxychloroquine usage in United States veterans hospitalized with Covid-19.
676 *medRxiv*, 2020.2004.2016.20065920
677 Mahevas, M., Tran, V.-T., Roumier, M., Chabrol, A., Paule, R., Guillaud, C., Gallien, S., Lepeule, R.,
678 Szwebel, T.-A., Lescure, X., *et al.* (2020). No evidence of clinical efficacy of hydroxychloroquine in
679 patients hospitalized for COVID-19 infection with oxygen requirement: results of a study using
680 routinely collected data to emulate a target trial. *medRxiv*, 2020.2004.2010.20060699
681 McGonagle, D., Sharif, K., O'Regan, A., and Bridgewood, C. (2020). The Role of Cytokines
682 including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-
683 Like Disease. *Autoimmun Rev*, 102537
684 Mehta, P., McAuley, D.F., Brown, M., Sanchez, E., Tattersall, R.S., Manson, J.J., and Hllh Across
685 Speciality Collaboration, U.K. (2020). COVID-19: consider cytokine storm syndromes and
686 immunosuppression. *Lancet* 395, 1033-1034
687 Mimori, S., Okuma, Y., Kaneko, M., Kawada, K., Hosoi, T., Ozawa, K., Nomura, Y., and Hamana,
688 H. (2012). Protective effects of 4-phenylbutyrate derivatives on the neuronal cell death and
689 endoplasmic reticulum stress. *Biol Pharm Bull* 35, 84-90

690 Mizutani, T., Fukushi, S., Saijo, M., Kurane, I., and Morikawa, S. (2004). Phosphorylation of p38
691 MAPK and its downstream targets in SARS coronavirus-infected cells. *Biochem Biophys Res*
692 *Commun* 319, 1228-1234
693 Osowski, C.M., and Urano, F. (2011). Measuring ER stress and the unfolded protein response using
694 mammalian tissue culture system. *Methods Enzymol* 490, 71-92
695 Ozcan, U., Yilmaz, E., Ozcan, L., Furuhashi, M., Vaillancourt, E., Smith, R.O., Gorgun, C.Z., and
696 Hotamisligil, G.S. (2006). Chemical chaperones reduce ER stress and restore glucose homeostasis in
697 a mouse model of type 2 diabetes. *Science* 313, 1137-1140
698 Parsons, K.S., Hsu, A.C., and Wark, P.A. (2014). TLR3 and MDA5 signalling, although not
699 expression, is impaired in asthmatic epithelial cells in response to rhinovirus infection. *Clin Exp*
700 *Allergy* 44, 91-101
701 Pathinayake, P.S., Hsu, A.C., Waters, D.W., Hansbro, P.M., Wood, L.G., and Wark, P.A.B. (2018).
702 Understanding the Unfolded Protein Response in the Pathogenesis of Asthma. *Front Immunol* 9, 175
703 Reid, A.T., Nichol, K.S., Chander Veerati, P., Moheimani, F., Kicic, A., Stick, S.M., Bartlett, N.W.,
704 Grainge, C.L., Wark, P.A.B., Hansbro, P.M., *et al.* (2020). Blocking Notch3 Signaling Abolishes
705 MUC5AC Production in Airway Epithelial Cells from Individuals with Asthma. *American journal of*
706 *respiratory cell and molecular biology* 62, 513-523
707 Saito, T., and Gale, M., Jr. (2008). Differential recognition of double-stranded RNA by RIG-I-like
708 receptors in antiviral immunity. *The Journal of experimental medicine* 205, 1523-1527
709 Shen, J., Snapp, E.L., Lippincott-Schwartz, J., and Prywes, R. (2005). Stable binding of ATF6 to BiP
710 in the endoplasmic reticulum stress response. *Molecular and cellular biology* 25, 921-932
711 Sheridan, B.C., McIntyre, R.C., Meldrum, D.R., and Fullerton, D.A. (1997). Pentoxifylline treatment
712 attenuates pulmonary vasomotor dysfunction in acute lung injury. *J Surg Res* 71, 150-154
713 Sullivan, R.J., Infante, J.R., Janku, F., Wong, D.J.L., Sosman, J.A., Keedy, V., Patel, M.R., Shapiro,
714 G.I., Mier, J.W., Tolcher, A.W., *et al.* (2018). First-in-Class ERK1/2 Inhibitor Ulixertinib (BVD-523)
715 in Patients with MAPK Mutant Advanced Solid Tumors: Results of a Phase I Dose-Escalation and
716 Expansion Study. *Cancer Discov* 8, 184-195
717 Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding, H.P., and Ron, D. (2000).
718 Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase
719 IRE1. *Science* 287, 664-666
720 Vanders, R.L., Hsu, A., Gibson, P.G., Murphy, V.E., and Wark, P.A.B. (2019). Nasal epithelial cells
721 to assess in vitro immune responses to respiratory virus infection in pregnant women with asthma.
722 *Respiratory research* 20, 259
723 Versteeg, G.A., van de Nes, P.S., Bredenbeek, P.J., and Spaan, W.J. (2007). The coronavirus spike
724 protein induces endoplasmic reticulum stress and upregulation of intracellular chemokine mRNA
725 concentrations. *Journal of virology* 81, 10981-10990
726 Walters, M.S., Gomi, K., Ashbridge, B., Moore, M.A., Arbelaez, V., Heldrich, J., Ding, B.S., Rafii,
727 S., Staudt, M.R., and Crystal, R.G. (2013). Generation of a human airway epithelium derived basal
728 cell line with multipotent differentiation capacity. *Respiratory research* 14, 135
729 Wang, G., Pang, Z., Chen-Yu Hsu, A., Guan, X., Ran, N., Yuan, Y., Wang, Z., Guo, Y., Zheng, R.,
730 and Wang, F. (2019). Combined treatment with SB203580 and dexamethasone suppresses non-
731 typeable Haemophilus influenzae-induced Th17 inflammation response in murine allergic asthma.
732 *European journal of pharmacology* 862, 172623
733 Wang, J.P., Bowen, G.N., Padden, C., Cerny, A., Finberg, R.W., Newburger, P.E., and Kurt-Jones,
734 E.A. (2008). Toll-like receptor-mediated activation of neutrophils by influenza A virus. *Blood* 112,
735 2028-2034
736 Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z., Lu, G., Qiao, C., Hu, Y., Yuen, K.Y., *et*
737 *al.* (2020a). Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. *Cell*
738 Wang, X., Xu, W., Hu, G., Xia, S., Sun, Z., Liu, Z., Xie, Y., Zhang, R., Jiang, S., and Lu, L. (2020b).
739 SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion. *Cell Mol*
740 *Immunol*
741 Wesselborg, S., Bauer, M.K., Vogt, M., Schmitz, M.L., and Schulze-Osthoff, K. (1997). Activation of
742 transcription factor NF-kappaB and p38 mitogen-activated protein kinase is mediated by distinct and
743 separate stress effector pathways. *The Journal of biological chemistry* 272, 12422-12429

744 Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang,
745 C.L., *et al.* (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin.
746 *Nature* 579, 270-273

747

Figure 1

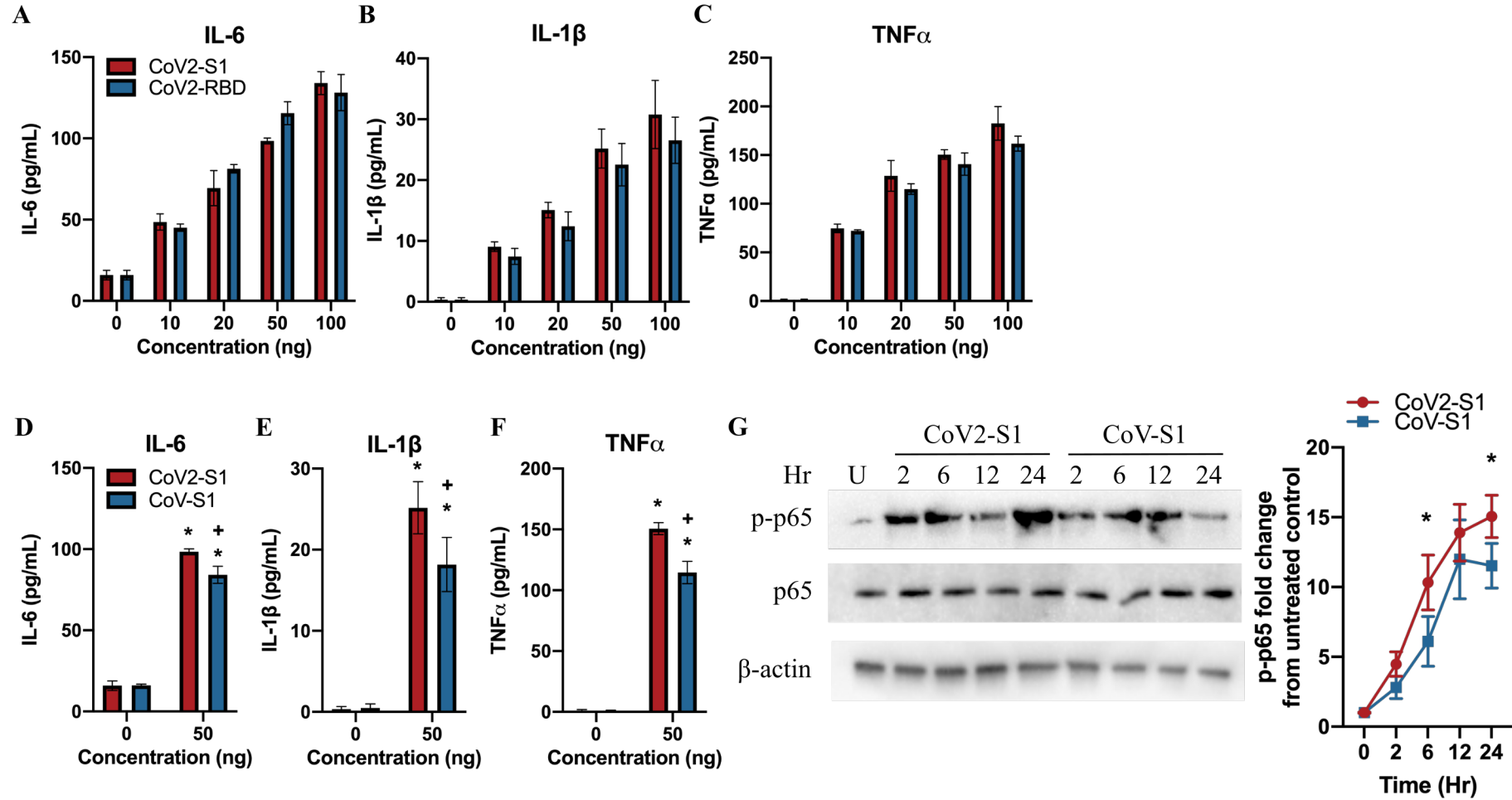


Figure 2

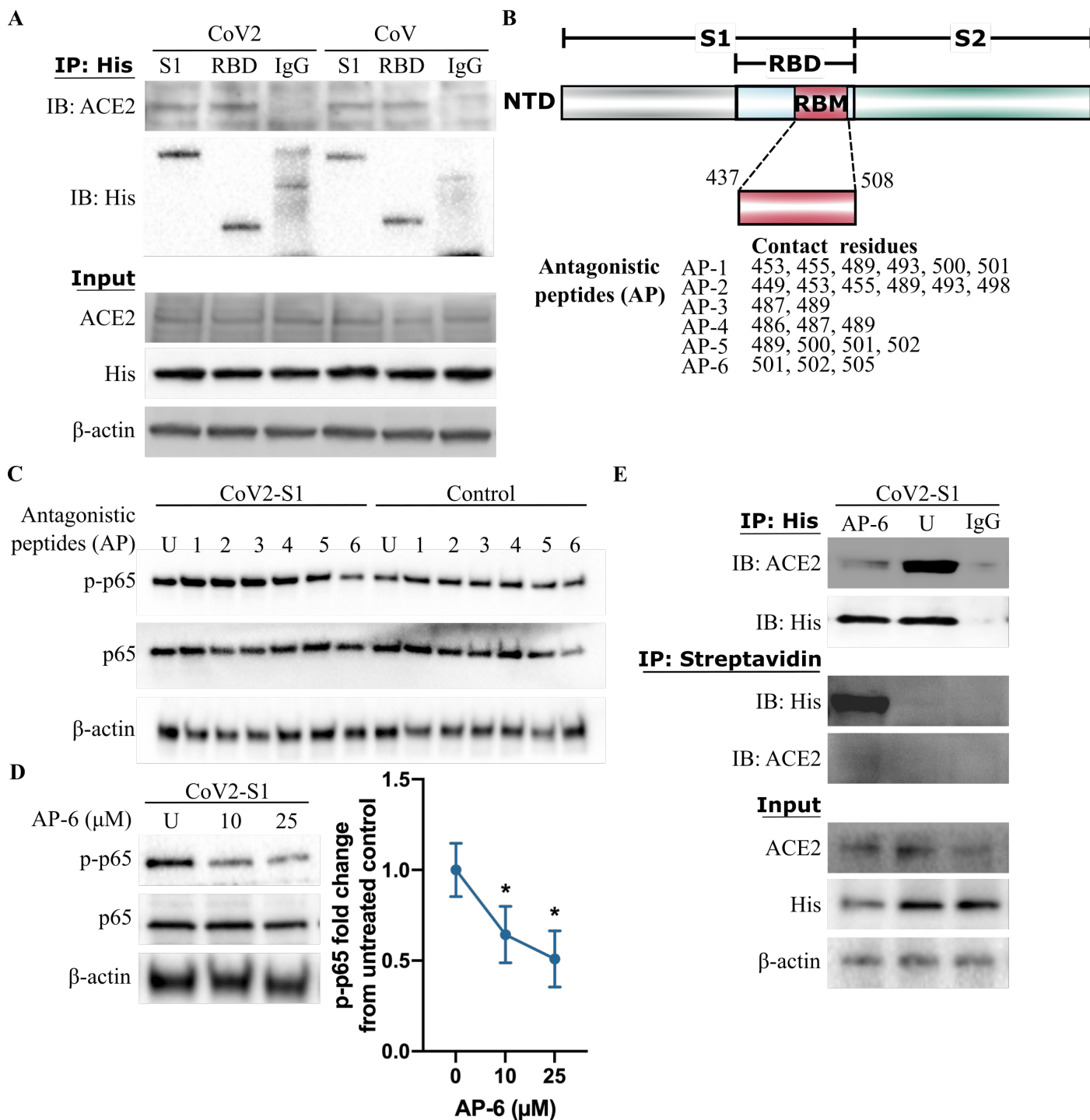
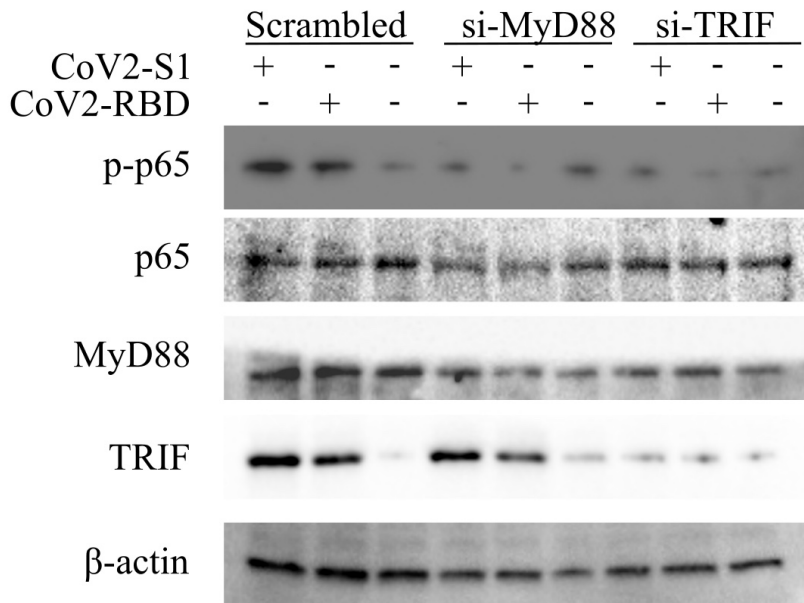
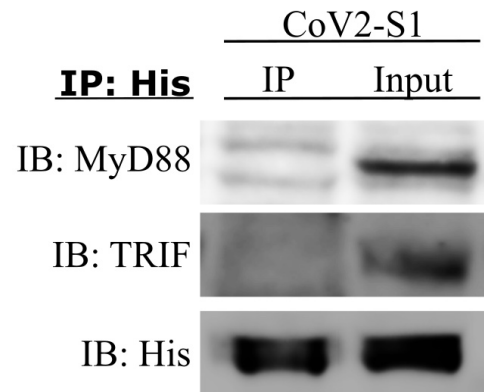


Figure 3

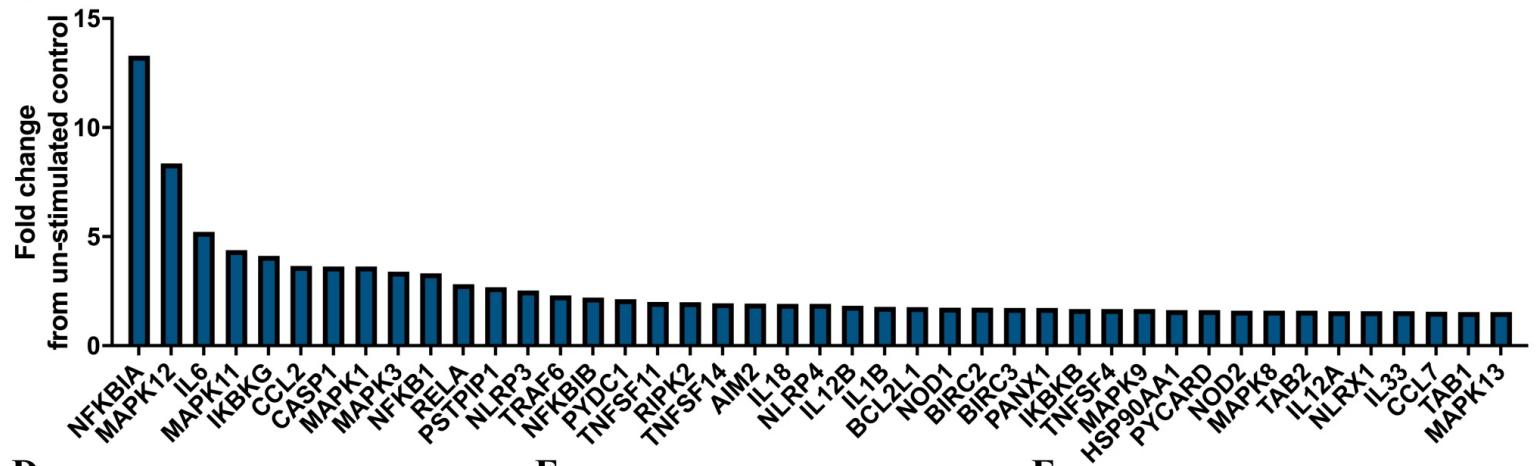
A



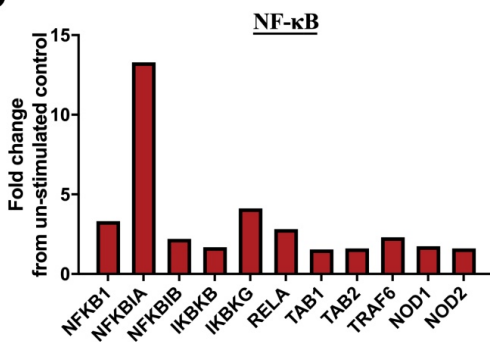
B



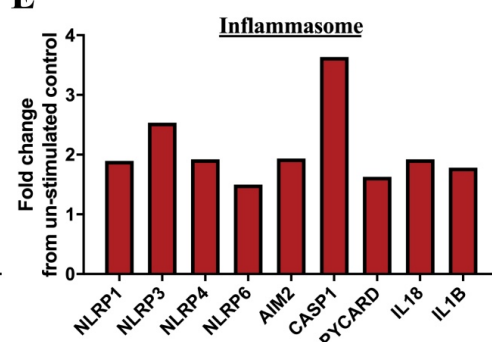
C



D



E



F

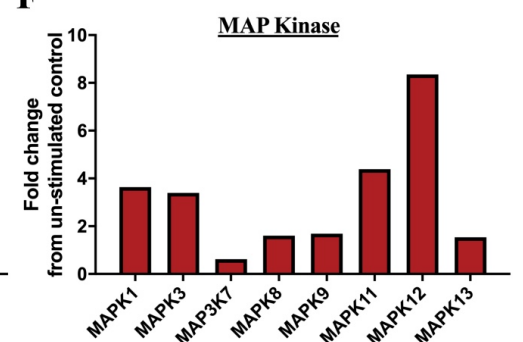
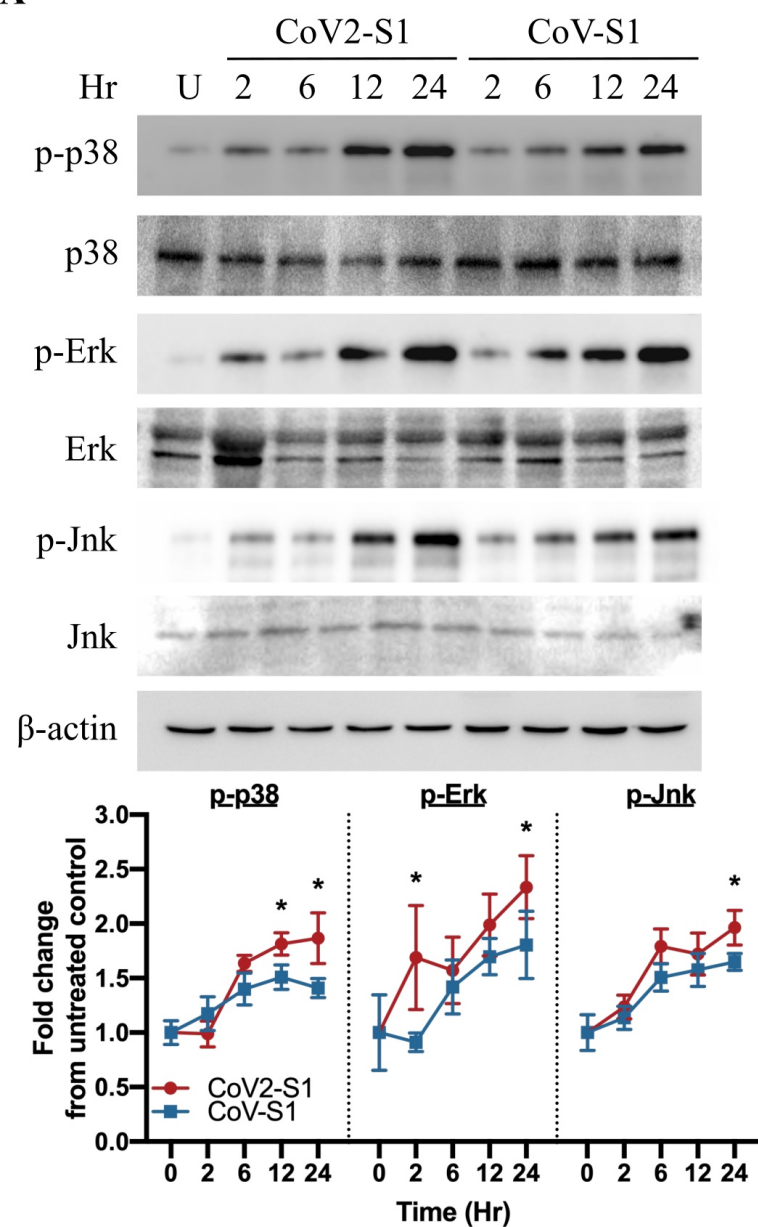


Figure 4

A



B

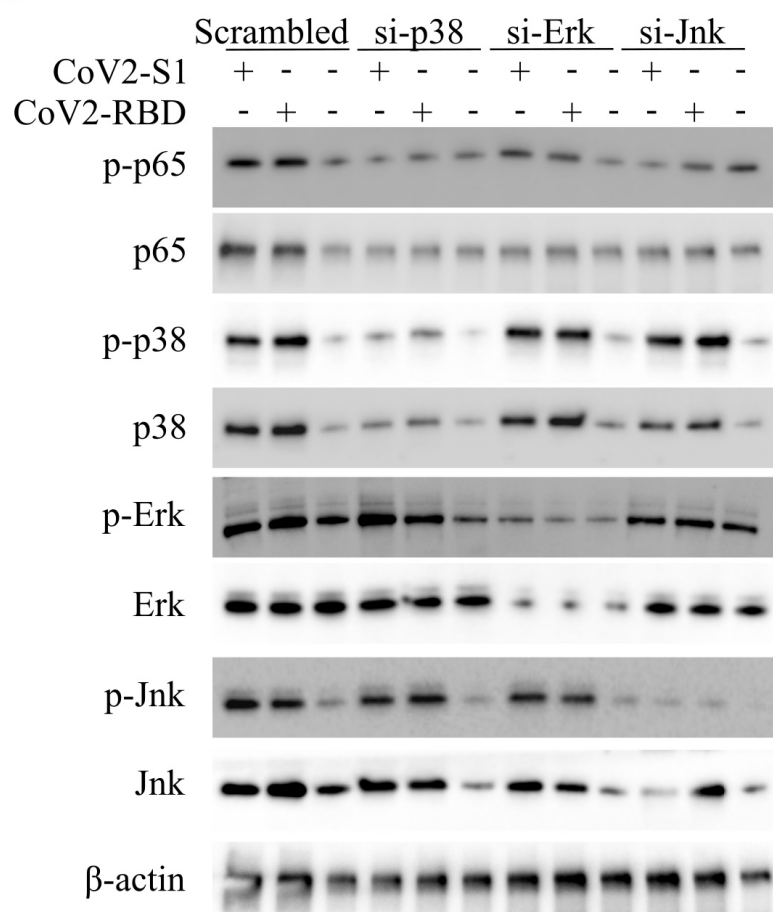
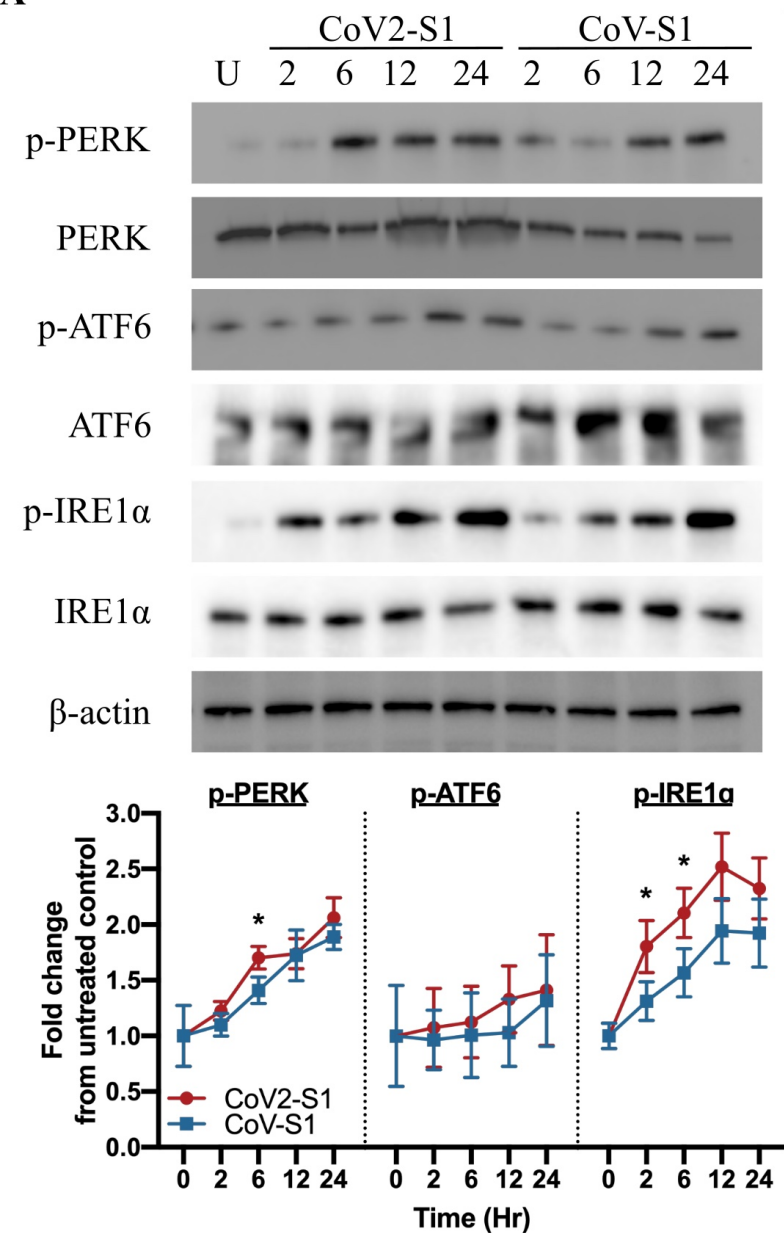
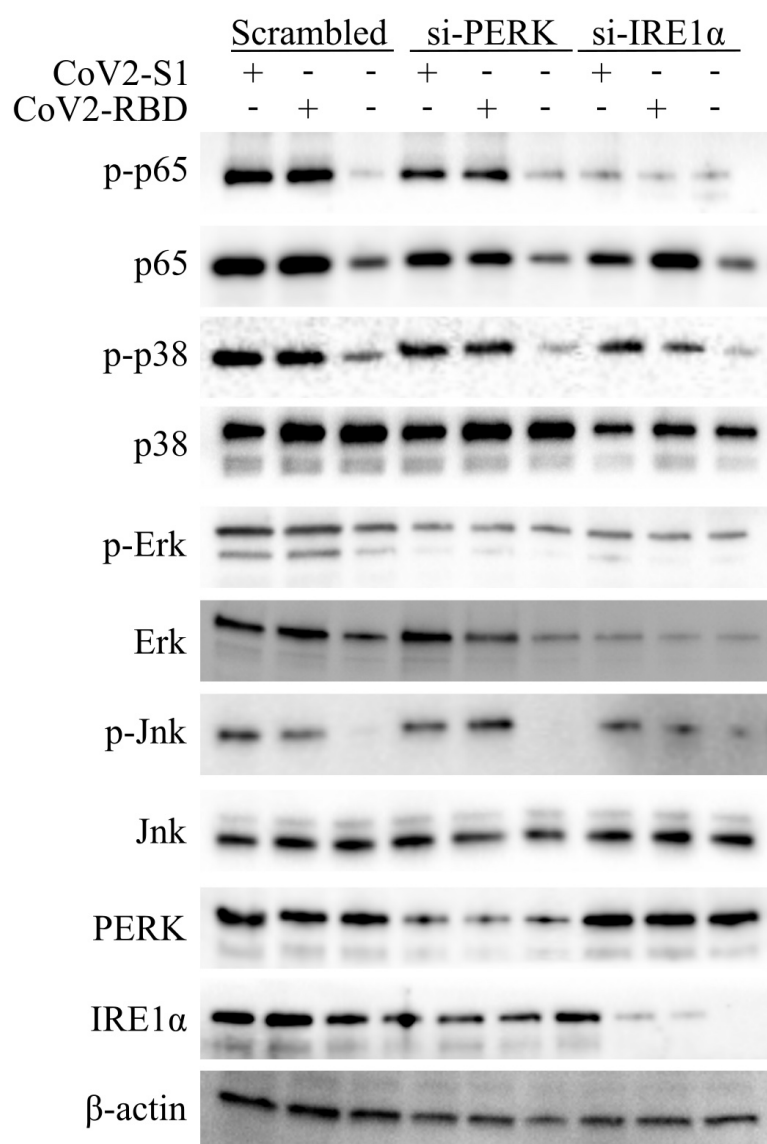


Figure 5

A



B



C

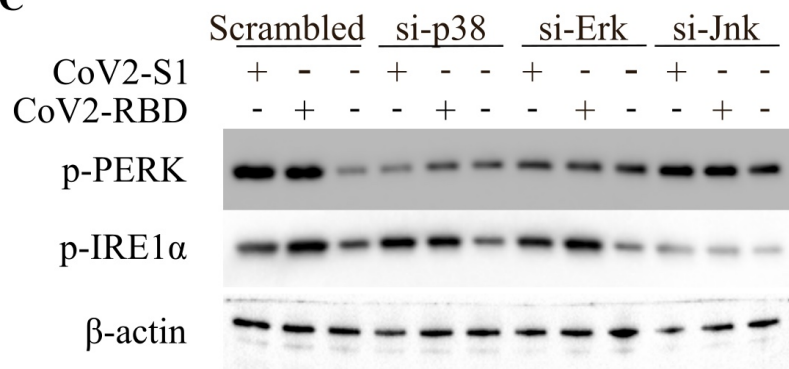


Figure 6

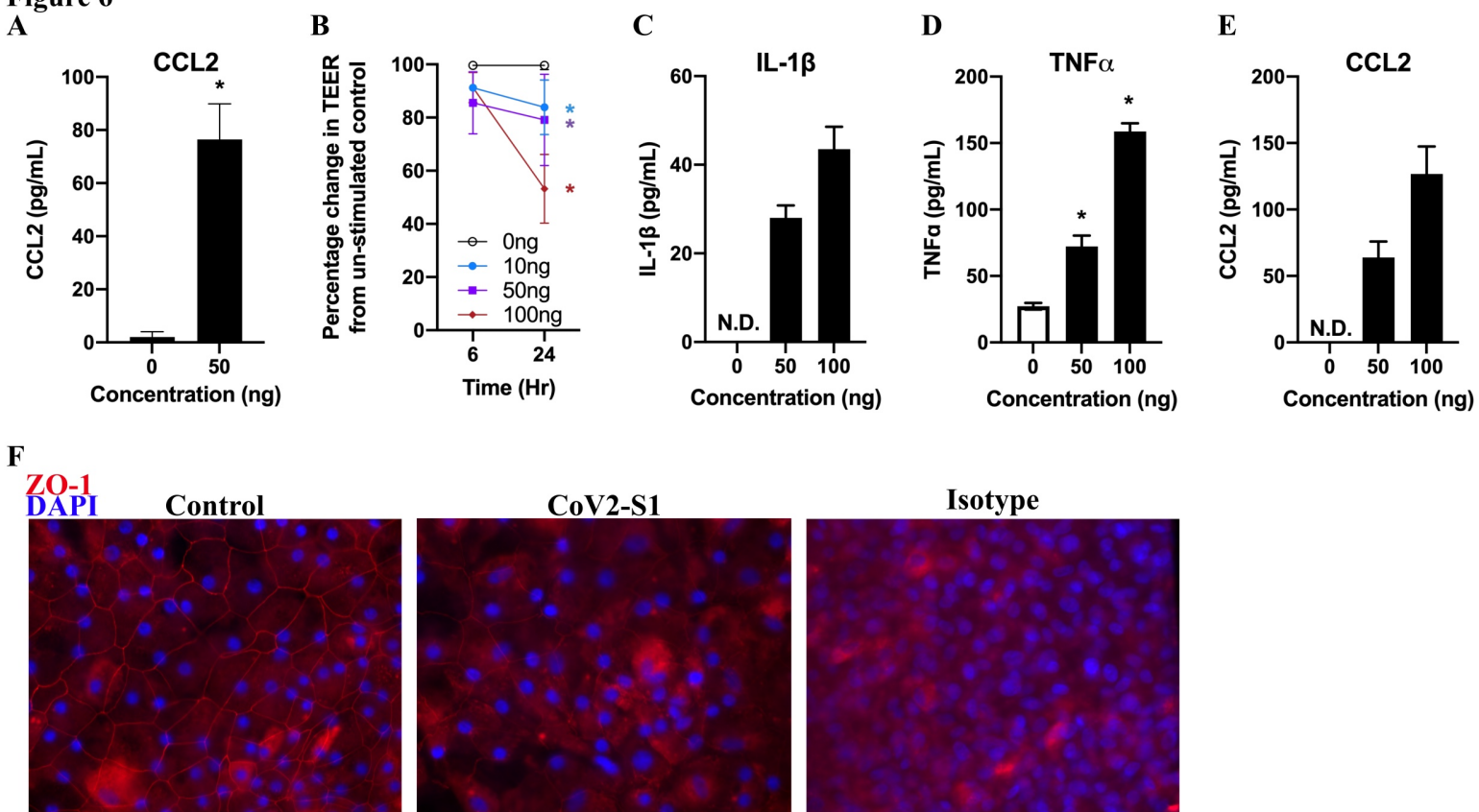


Figure 7

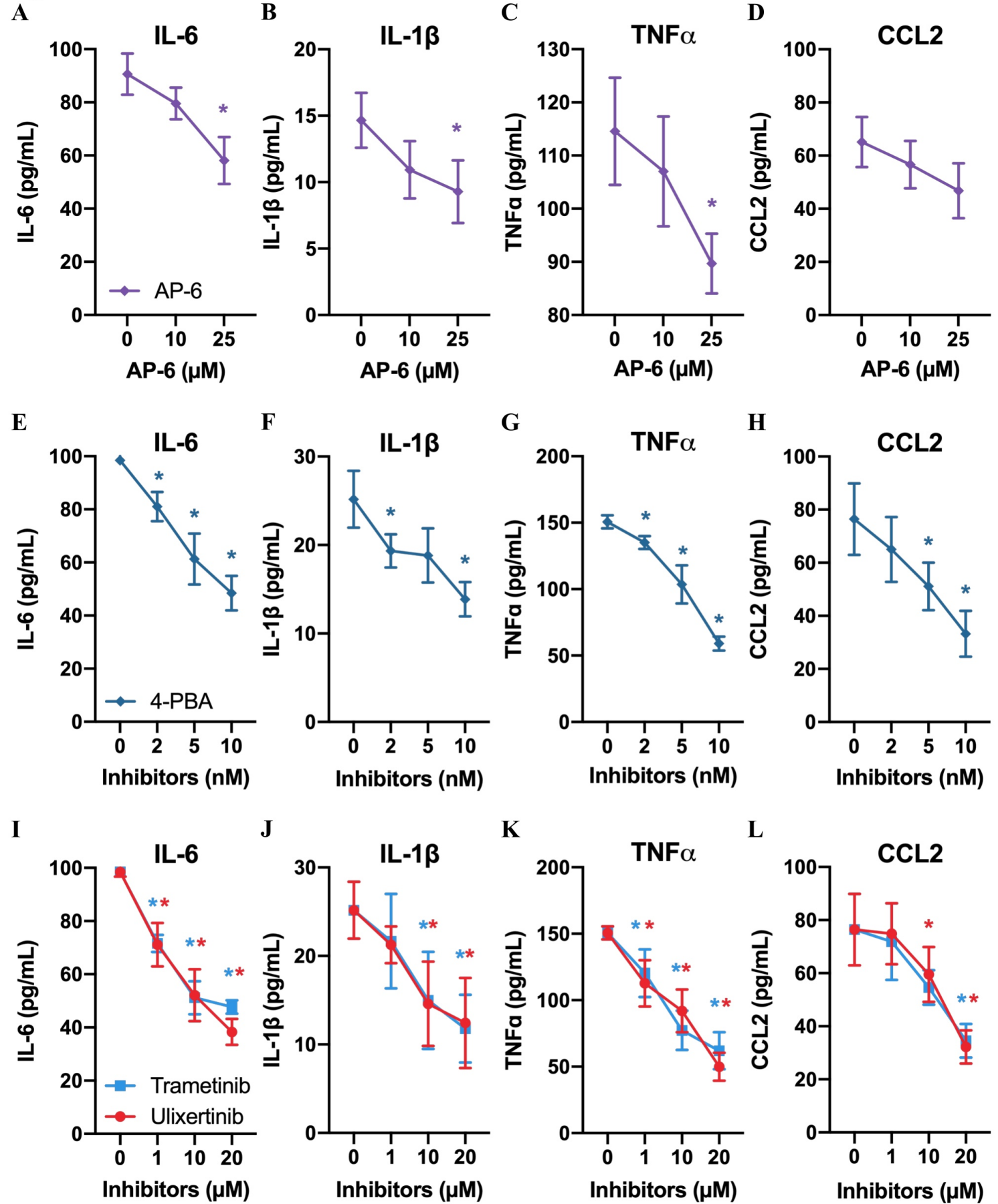


Figure 8

